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Human Immunodeficiency Virus Type 2 (HIV-2) in Portugal: Clinical Spectrum, Circulating Subtypes, Virus Isolation, and Plasma Viral Load

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The human immunodeficiency virus type 2 (HIV-2) is responsible for 4.5% of AIDS cases in Portugal. Six HIV-2 subtypes have been described so far, subtype A being proposed as more pathogenic than the rest. The relationship between the clinical status and levels of both cellular and plasma HIV-2 viraemia is not well known, nor their modifications under antiretroviral therapy. Thirty-two consecutive HIV-2 infected persons (17 men, 15 women) attending two different hospitals in Lisbon in 1997 were enrolled prospectively in the study. All but 4 individuals most likely acquired the infection through heterosexual contact. More than half of the study population was of African origin, mainly from Guinea-Bissau. Eleven (34.4%) patients had developed clinical manifestations included within the B or C groups of the CDC classification system for HIV infection, with the rest being asymptomatic. Half of the population was undergoing antiretroviral treatment at the time of the study. HIV-2 subtypes were investigated using a new *Nef*-based restriction fragment length polymorphism (RFLP) method that allows differentiation of the main two variants, A and B. Plasma viral load was quantified using a new quantitative competitive reverse transcriptase polymerase chain reaction (QcRT-PCR) procedure as well as the Amp-RT assay. Virus isolation was attempted from peripheral blood mononuclear cells. All but one person carried HIV-2 subtype A. Plasma viraemia examined by QcRT-PCR was measurable in 15 (50%) of 30 subjects, yielding in all instances values below 20,000 HIV-2 RNA copies per ml. Plasma RT activity could be detected in only 10 (33%) of 30 subjects, a rate much lower than that seen in HIV-1 infection. Virus was isolated from 16 (53.3%) of 30 patients. A significant correlation was found between CD4+ counts, clinical status, rate of virus isolation, and plasma viral load by both QcRT-PCR and Amp-RT. In conclusion, HIV-2 subtype A is the predominant variant circulating in Portugal among both natives and immigrants. A lower cellular and plasma viral load with respect to HIV-1 was seen in persons without immunosuppression, from whom the rate of virus recovery was extremely low. ***J. Med. Virol. 61:111–116, 2000.***

KEY WORDS: HIV-2; subtypes; viraemia; epidemiology; Portugal

INTRODUCTION

The human immunodeficiency virus type 2 (HIV-2) was identified in West Africa in 1986 [Clavel et al., 1986]. It has 50% nucleotide homology with HIV-1 [Guyader et al., 1987]. HIV-2 infected individuals exhibit a slower disease progression and relatively lower transmissibility in comparison to HIV-1 [Marlink et al., 1994; Adjorlolo-Johnson et al., 1994; Marlink, 1996]. Among the pathogenetic mechanisms contributing to the lower virulence of HIV-2, a reduced functionality of its *nef* regulatory gene [Switzer et al., 1998] and a low infectious virus level in infected individuals, have been

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suggested [Simon et al., 1993]. Six different genetic subtypes of HIV-2 (A–F) have been recognized so far, HIV-2 subtype A being the most predominant [Gao et al., 1994; Chen et al., 1997; Takehisa et al., 1997; Peeters et al., 1998]. Lower or even lack of pathogenicity have been suggested for non-A subtypes [Gao et al., 1994]. Knowledge about the biological characteristics and the epidemic spread of the different HIV-2 subtypes is still poor. It has been very difficult to undertake large scale comparative studies of subtypes because of the lack of simple, quick, and cheap tests for the recognition of HIV-2 variants. Although sequence analysis remains the most accurate genetic approach for the characterisation of viral genomes, other less expensive and labour intensive genetic techniques could be helpful for epidemiological purposes.

HIV-2 has been found to be quite common in West African countries and, to a lesser extent, in other regions with historical socioeconomic ties, such as the former Portuguese colonies, including Angola, Mozambique, Guinea-Bissau, India, and Brazil [Santos-Ferreira et al., 1990; Kanki et al., 1994; Marlink et al., 1996; Heredia et al., 1998a; Kanki, 1999]. In Europe and North America, most reported HIV-2-infected subjects have been African immigrants or, less frequently, natives who had lived in West Africa for a while or who had had sex with people from that region [Smallman-Raynor and Cliff, 1991; O'Brien et al., 1992; Quinn, 1994; Van der Ende et al., 1996; Soriano et al., 1996].

Portugal has a high number of HIV-2 infections and serves as a diffusion node for the virus within Europe [Smallman-Raynor and Cliff, 1991]. The presence of the virus in Portugal has been traced to the 1970s or even earlier [Saimot et al., 1987; Bryceson et al., 1988]. Currently, up to 4.5% of AIDS cases in Portugal are caused by HIV-2, and epidemiological surveys show that more than 60% of the new HIV-2 infections are among the indigenous population and cannot be linked directly to African contacts [Quinn, 1994; Ministério de Saúde, 1999].

In this study the prevalence of different circulating subtypes, their main virological features and its relation with the clinical and immunological status were studied in a group of HIV-2-infected subjects living in Portugal.

MATERIALS AND METHODS

Subjects

Thirty-two consecutive HIV-2 seropositive individuals in follow-up studies in two hospitals located in Lisbon were recruited prospectively in the study in 1997. All patients were informed and consented to participate in the study. Demographics and clinical status were recorded during the last visit and whole blood was drawn in EDTA tubes. Peripheral blood mononuclear cells (PBMCs) and plasma were prepared by Ficoll-Hypaque gradient centrifugation, and cryopreserved at −70°C until used. Cell number and viability were determined by the Trypan blue dye exclusion method. To confirm HIV-2 infection (and exclude co-infection with HIV-1) all sera were tested by dot blot with synthetic transmembrane peptides (Pepti-LAV, Sanofi-Diagnostics Pasteur).

HIV-2 Subtyping

To develop a more rapid and reliable assay to subtype all 32 HIV-2 samples, a sequence alignment was made of 20 HIV-2 *nef* genes (5 subtype B, 15 subtype A) available at the GenBank. *Nef* sequences of subtypes other than A and B were not available for restriction map analysis. A *DsaI* restriction site was present in all subtype B sequences, but in none of the A subtypes. Thus, this endonuclease was selected for use in an RFLP-based method to differentiate between subtypes A and B.

Cryopreserved PBMCs were lysed at a concentration of 4–6 × 10^6 cells per ml in PCR lysis buffer supplemented with 60 μg of proteinase K per ml at 56°C for 1 hr. The proteinase K was then inactivated by boiling for 15 min, and the PCR lysates were stored at −20°C. Fifty microliters of PCR lysates were used in first-round amplification of the *nef* sequences using 1 min for template denaturation at 94°C, 1 min of primer annealing at 41°C, and 1 min of primer extension at 72°C for 40 cycles of PCR. The consensus oligomers 2NEFFD (5′ GGG CTA TAG GCC (T/A)GT (T/A)TT CTC (T/C)TC CCC 3′) and SLTR5 (5′ ACC TGC TAG TGC TGG AGA GAA CCT 3′) were used to amplify a 1299-bp sequence encompassing the full *nef* gene [Switzer et al., 1998]. Five microliters of the first-round amplification product were used in a nested PCR assay to amplify a 583-bp sequence using the consensus internal oligomers 2NEFSF2 (5′ GGG GAC TGG AAG GG(A/C) TGT (T/A)TT A(C/T)A 3′) and NEF9 (5′ CCT CTC CGC AGA GCG ACT GAA TAC A 3′). *DsaI* digestion of the amplified *nef* fragment from HIV-2 subtype B specimens generates fragments of 240-bp and 343-bp in size, whereas samples carrying subtype A strains are not digested with the *DsaI* restriction endonuclease.

Sequence analysis of *nef* and *pol* gene fragments was carried out using an automatic sequencer (Applied Biosystems, Madrid, Spain) to confirm the genotyping results of the RFLP method.

Quantitation of HIV-2 Plasma Viraemia by QcRT-PCR

A home-made quantitative-competitive reverse transcriptase PCR-based assay (QcRT-PCR) was used to quantify plasma HIV-2 RNA molecules. The amplified products were detected using an enzyme immunoassay (EIA) [Gomes et al., 1998]. An internal control ($RNApg_3$) was constructed to control all the amplification steps [Clementi et al., 1996]. This internal control is amplified by the same pair of primers of the wild type and the amplified fragment has almost the same size. They differ in 72 bp that allowed the design of two different probes, one for HIV-2 wild type (pHIV) and another for the internal control (pPhi).

HIV-2 RNA extraction was carried out from 200 μl of plasma, using the RNAgents Total Isolation System

Kit (Promega). QcRT-PCR amplifications were carried out in 50 μl reaction mixtures containing 10 μl of the RNA sample, that was co-amplified in 3 parallel reactions each containing 100, 1000, or 10000 copies of $RNApg_3$, primers TM1 (5′-biotin) positions 7614–7636 in HIV-2ROD and TM2 positions 7974–7950 at 0.2 μM each, using the Access RT-PCR System Kit (Promega). Cycling conditions were 48°C for 45 min, 95°C for 30 sec, 62°C for 60 sec, and 72°C for 90 sec [Gomes et al., 1998].

Amplified products were detected by using an EIA-based method. Briefly, amplified products were added to two parallel microplate wells coated with streptavidin, and incubated for 2 hr at 37°C. DNA was then denatured by adding NaOH and incubated for 10 min at room temperature. Plates were washed with 5× SSPE. For hybridization, a pHIV probe labelled with digoxigenin was added to one of the plate wells and the internal standard probe, also labelled with digoxigenin (pPhi), was added to the other well, and incubated for 3 hr at 42°C. After 4 washes with 5× SSPE, plates were incubated for 1 hr at 37°C with 100 μl of a 1:5000 dilution of anti-digoxigenin Fab conjugated with alkaline phosphatase. The final wash was with phosphate-buffered saline. A colourimetric reaction was obtained using PNPP as substrate, and the plates were read at 405 nm. Quantitation of the HIV-2 RNA copy number was performed by plotting the ratio between the ODs of the tested HIV-2 specimens and the $RNApg_3$ (Y axis) against predetermined RNA copy numbers of the internal standard (X axis). The copy numbers of the tested samples, expressed as number of copies per ml plasma, were calculated from the linear regression analysis when $Y = 1$. A pool of plasma from seronegative blood donors was used as the negative control. The detection limit of the assay was 100 HIV-2 RNA copies per ml [Gomes et al., 1998].

Viral Load Measurement by Amp-RT

Amp-RT is a PCR-based ultra-sensitive assay for measuring RT activity by using a known non-retroviral heteropolymeric RNA template and a complementary DNA oligonucleotide primer. The cDNA is detected by PCR amplification and probing with an internal oligonucleotide [Heneine et al., 1995; Yamamoto et al., 1996; García-Lerma et al., 1998, 1999]. For testing of plasma, a volume of 100 μl was clarified by centrifugation at 10,000 × *g* for 5 min and was then ultracentrifuged at a fixed angle at 99,000 × *g* for 1 hr at 4°C. The viral pellet was resuspended in 100 μl of RT buffer (50 mM Tris-Hcl, 50 mM KCl, 10 mM $MgCl_2$), and aliquots of 2 to 10 μl were used for analysis by the Amp-RT assay. RT levels were quantitated by ELISA, with a standard curve generated with known units of RT activity from a reference HIV-1 stock [García-Lerma et al., 1998]. The Amp-RT signals were expressed as units of RT activity per milliliter and reflect the average of duplicate results. Units of RT activity were converted into virion equivalents based on the knowledge of the amount of RT activity per one HIV-1 particle estimated testing a HIV-1 reference control virus [García-Lerma et al., 1998]. The virion equivalent numbers represent estimations and should not be regarded as absolute values.

Virus Isolation

Patient's cryopreserved cells were incubated with uninfected donor PBMCs stimulated previously with phytohaemaglutinin [Clarck et al., 1991]. The tissue culture infective dose ($TCID/10^6$ cells) [Ho et al., 1989] was defined as the reciprocal of the highest dilution resulting in at least one culture well being antigen p24 positive.

Statistical Analysis

The results are expressed as percentage (%) of patients with respect to the total study population. The Chi square test (Yates correction when appropriate) was used to compare the rate of virus isolation and positive plasma viraemia values in persons with different clinical and immunological status.

RESULTS

Table I summarizes the main features of the study population. More than half were of African origin, mainly from Guinea-Bissau. Seventeen persons (53.1%) were male. Although all subjects were older than 14 years, age was known for only eleven (mean age: 45.9). All but 4 individuals most likely acquired the infection through heterosexual contacts. Half of the study population was receiving antiretroviral therapy at the time of enrollment in the study. Using the Centers for Diseases Control (CDC) classification system for HIV [CDC, 1992], minor (CDC B) or major (CDC C) clinical manifestations had been diagnosed in 11 (34.4%) subjects. The remaining 21 persons had been asymptomatic (CDC A) up to the time of the study, although 10 of them already had CD4+ counts below 500 cells/μl (CDC A.2 and A.3).

Samples from all but one individual carried HIV-2 subtype A. The carrier of the subtype B was a 45-year-old white male, born in Lisbon, who had been in the Ivory Coast for two years (1979–1980), where he reported several heterosexual contacts. He developed AIDS in 1997. Sequence analysis of *nef* or *pol* gene regions from this patient, as well as from another six persons with subtype A viruses, confirmed the results provided by RFLP.

Plasma viraemia examined by QcRT-PCR was measurable in only 15 (50%) of 30 subjects, yielding in all instances values below 20,000 HIV-2 RNA copies per ml. Because the probes for quantitation in the described QcRT-PCR method are added at the stage of amplification, a low efficiency in RNA recovery could explain the low sensitivity of the assay. Plasma RT activity could be detected in only 10 (33%) of 30 subjects, however, a rate that is lower than that seen in HIV-1-infected patients [CDC, 1992]. Virus was isolated from 16 (53.3%) of 30 patients.

Table II shows the interrelationship between the

TABLE I. Demographics of the Study Population and Virological Results*

Number	Country of infection	Race	Gender	Route of infection	CD4 count (cells/μl)	CDC	Antiretroviral therapy	Virus isolation	$TCID_{50}$	Subtype	Amp-RT (number virion/ml)	QcRT-PCR (number RNA copies/ml)
1	Guinea-Bissau	Black	Male	Htsex	205 (15%)	A.2	No	Pos	0.5	A	ND	1,137
2	Guinea-Bissau	Black	Male	Htsex	10 (3%)	C.3	ZDV, ddI	Pos	5	A	3,282	Neg
3	Mozambique	White	Male	Htsex	143 (14%)	A.2	No	Pos	5	A	146,622	18,247
4	Guinea-Bissau	Black	Male	Htsex	624 (31%)	A.1	No	Pos	Neg	A	Neg	Neg
5	Guinea-Bissau	Black	Female	Htsex	340 (26%)	A.2	ZDV, ddI	Pos	Neg	A	Neg	3,202
6	Guinea-Bissau	Black	Female	Htsex	187 (25%)	B.3	ZDV, 3TC	Pos	5	A	1,964	1,559
7	Portugal	White	Male	Htsex	282 (25%)	B.2	No	Neg	Neg	A	Neg	3,499
8	Portugal	White	Female	Transfusion	832 (48%)	A.1	No	Neg	Neg	A	Neg	Neg
9	Guinea-Bissau	Black	Male	Htsex	1033 (49%)	A.1	No	Neg	Neg	A	Neg	Neg
10	Guinea-Bissau	Black	Female	Htsex	657 (31%)	A.1	No	Neg	Neg	A	Neg	Neg
11	Guinea-Bissau	Black	Female	Htsex	917 (32%)	A.1	No	Neg	Neg	A	Neg	11,875
12	Portugal	White	Male	Htsex	20 (6%)	C.3	ddI, 3TC	Neg	Neg	A	Neg	Neg
13	Portugal	White	Male	Htsex	282 (21%)	A.2	ZDV, ddI	Pos	Neg	A	Neg	Neg
14	Portugal	White	Female	Htsex	717 (35%)	A.1	No	Pos	Neg	A	Neg	Neg
15	Guinea-Bissau	Black	Male	Htsex	566 (38%)	A.2	ZDV	Neg	Neg	A	Neg	1,084
16	Guinea-Bissau	Black	Male	Transfusion	323 (33%)	B.2	ZDV, 3TC	Pos	0.5	A	Neg	Neg
17	Guinea-Bissau	Black	Male	Htsex	90 (6%)	B.3	ddI, 3TC	Pos	Neg	A	1,783	Neg
18	Cape Verde	Black	Female	Htsex	71 (25%)	A.3	ZDV, 3TC	Pos	ND	A	2,668	1,812
19	Guinea-Bissau	Black	Female	Htsex	170 (17%)	C.3	ZDV	Pos	500	A	1,964	7,254
20	Guinea-Bissau	Black	Male	Htsex	173 (24%)	A.2	ZDV, ddI	Pos	50	A	2,239	6,160
21	Cape Verde	Black	Male	Htsex	168 (4%)	B.2	ZDV, ddC	ND	ND	A	671,738	ND
22	Mozambique	White	Male/51	Htsex	918	A.1	No	Neg	Neg	A	Neg	Neg
23	Ivory Coast	White	Male/45	Htsex	165	C.3	ZDV, 3TC, IDV	Neg	Neg	B	8,896	2,394
24	Portugal	White	Female/58	Htsex	308	A.2	ZDV, ddI	Pos	5	A	Neg	Neg
25	Portugal	White	Female/36	Htsex	446	A.2	No	Pos	5	A	Neg	Neg
26	Portugal	White	Female/61	Htsex	1446	A.1	No	Neg	Neg	A	Neg	Neg
27	Portugal	White	Male/67	Htsex	973	A.1	No	Neg	Neg	A	Neg	2,165
28	Portugal	White	Female/52	Htsex	600	A.1	No	Neg	ND	A	Neg	6,181
29	Portugal	White	Female/46	Htsex	763	A.1	No	Neg	Neg	A	Neg	2,943
30	Guinea-Bissau	Black	Male/44	Htsex	197	C.3	ZDV, 3TC, IDV	Pos	50	A	8,633	18,822
31	Guinea-Bissau	Black	Female/15	Htsex	87	C.3	D4T, 3TC, IDV	Neg	0.5	A	Neg	Neg
32	Portugal	White	Female/30	IDU & Htsex	246	A.2	No	ND	ND	A	ND	ND

*CDC, Centers for Disease Control; Htsex, heterosexual; IDU, injecting drug user; ddI, didanosine; ZDV, zidovudine; 3TC, lamivudine; D4T, stavudine; ddC, zalcitabine; IDV, indinavir.

TABLE II. Interrelationship Between Virological Features, CDC Status, and Antiretroviral Treatment in HIV-2-Infected Persons*

	N	CDC A.1	CDC A.2 and A.3	CDC B and C
Study population	32	11	10	11
Antiretroviral therapy	16	0	6	10
Detectable plasma viremia (QcRT-PCR and/or Amp-RT	18/31 (58.1%)	4/11 (36.4%)	6/9 (66.7%)	8/10 (80%)
Positive virus isolation from PBMCs	16/30 (50%)	2/11 (18.2%)	8/9 (88.9%)	6/10 (60%)

*QcRT-PCR, quantitative competitive reverse transcriptase polymerase chain reaction; Amp-RT, Amplification reverse transcriptase assay; PBMCs, peripheral blood mononuclear cells.

clinical status, the concurrent administration of antiretroviral therapy, and the main virological features, such as the plasma viral load and the ability to grow in virus culture. It is noteworthy that asymptomatic subjects with CD4+ counts above 500 cells/μl (CDC A.1) showed a significantly lower plasma viraemia, either by QcRT-PCR or Amp-RT with respect to individuals with symptomatic disease or CD4+ counts below 500 cells/μl (36.4% vs. 73.7%; $P = 0.01$). Similarly, the rate of virus recovery in culture was significantly lower in persons at CDC A.1 with respect to the rest, that have symptoms (CDC B or C) or less than 500 CD4+ cells/μl (18.2% vs. 73.7%; $P = 0.02$). These results were obtained despite 76.2% (16 out of 21) of HIV-2-infected subjects having a past history of HIV-associated illnesses or CD4+ counts below 500 cells/μl and who were undergoing antiretroviral therapy at the time their blood was screened.

DISCUSSION

Clinical and virological data are described from 32 HIV-2-infected individuals living in Portugal. All study participants except four had probably been infected through heterosexual contact. Eleven patients had already presented with clinical manifestations linked to HIV infection (CDC Groups B and C). More than half were of African origin, mainly from Guinea-Bissau, a former Portuguese colony, that seems to be the epicentre for HIV-2 infection. All individuals but one in this study were infected with HIV-2 subtype A. This variant is the most common worldwide, except in Ivory Coast, Ghana, and Equatorial Guinea [Takehisa et al., 1997; Heredia et al., 1997; Machuca et al., 1998; Cavaco-Silva et al., 1998; Machuca et al., 1999], where HIV-2 subtype B is prevalent. Because these regions are not former colonies of Portugal, it was not surprising that subtype A was the most prevalent in our study population, in agreement with recent reports from Portugal [Cavaco-Silva et al., 1998; Heredia et al., 1998b]. Because no data were available in most instances on the duration of HIV-2 infection, no conclusions can be drawn on the pathogenicity of subtype A: infected subjects in our series included both symptomatic and healthy individuals. Moreover, the single subject carrying subtype B developed AIDS after an estimated period of infection of 17 years. This observation supports the theory that subtype B can be pathogenic like subtype A. Finally, the *nef*-based RFLP described above allowed the differentiation of the main HIV-2 subtypes, A and B, in all instances. Thus, this RFLP method represents a useful molecular technique for the rapid distinction of the main HIV-2 subtypes, and can be applied for epidemiological purposes.

The results of virus isolation studies, and plasma viral load were concordant with the clinical status. Virus isolation from PBMCs was successful in 73.7% of subjects with less than 500 CD4 cells/μl or belonging to CDC Groups B and C, but only in 18.2% of those belonging to CDC A.1. These numbers were similar for plasma viraemia, measured by either QcRT-PCR or Amp-RT (73.7% and 36.4%, respectively). This association between advanced disease stage and positive plasma viraemia was obtained despite more than three quarters of patients having symptomatic disease or low CD4+ counts and who were on antiretroviral therapy, that should reduce virus replication. Overall, the data are in agreement with the viral load results obtained by others [Albert et al., 1990; Simon et al., 1993; Albert et al., 1996], although this study is one of the few studies in which quantitation of HIV-2 plasma viraemia has been examined. The assessment of plasma viraemia in one study [Simon et al., 1993] was carried out by using a quantitative end-point dilution of viruses isolated from PBMCs, and a qualitative assay for recovering viruses directly from plasma. To our knowledge, our report is the first attempting the direct quantitation of HIV-2 particles in plasma, either measuring the number of HIV-2 RNA molecules by QcRT-PCR or the RT activity by the Amp-RT assay. Our data show that the concentration of virus particles in plasma from HIV-2-infected subjects is lower than that seen in HIV-1 carriers. This is true for all stages of the disease, being undetectable in most subjects with CD4+ counts greater than 500 cells/μl, and rising to values not higher than 20,000 HIV-2 RNA copies/μl per ml in symptomatic patients.

Differences in viral load throughout most of the natural history of HIV-1 and HIV-2 infections most likely account for the observed differences in the rate of disease progression and transmissibility between both viruses [De Cock et al., 1993; Simon et al., 1993; Ariyoshi et al., 1996; Kanki, 1999]. Further studies are needed to clarify whether the linear predictive value of plasma viraemia on clinical outcome established for

HIV-1 [Mellors et al., 1997] could apply in a similar fashion to HIV-2 infection, in which much lower levels of viraemia exist.

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REFERENCES

Adjorlolo-Johnson G, De Cock K, Ekpini E, et al. 1994. Prospective comparison of mother-to-child transmission of HIV-1 and HIV-2 in Abidjan, Ivory Coast. JAMA 272:462-466.

Albert J, Naucler A, Bötiger B, et al. 1990. Replicative capacity of HIV-1, like HIV-1, correlates with severity of immunodeficiency. AIDS 4:291–295.

Albert J, Stalhanske P, Marquina S. 1996. Biological phenotype of HIV type 2 isolates correlates with V3 genotype. AIDS Res Hum Retroviruses 12:821–828.

Ariyoshi K, Berry N, Wilkins A, et al. 1996. A community-based study of HIV-2 provirus load in a rural village in West Africa. J Infect Dis 173:245–248.

Bryceson A, Tomkins A, Ridley D. 1988. HIV-2-associated AIDS in the 1970s. Lancet ii:22.

Cavaco-Silva P, Taveira N, Rosado L, et al. 1998. Virological and molecular demonstration of HIV-2 vertical transmission. J Virol 72:3418–3422.

CDC. 1992. Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR 41 (RR-17):1–19.

Clarck S, Saag M, Decker W, et al. 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N Engl J Med 324:954–960.

Clavel F, Guétard D, Brun-Vézinet F, et al. 1986. Isolation of a new human retrovirus from West African patients with AIDS. Science 233:343–346.

Clementi M, Menzo S, Begnarelli P, et al. 1996. Clinical use of quantitative molecular methods in studying human immunodeficiency virus type 1 infection. Clin Microbiol Rev 9:135–147.

Chen Z, Luckay A, Sodora D, et al. 1997. Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys. J Virol 71:3953–3960.

De Cock K, Adjorlolo G, Ekpini E, et al. 1993. Epidemiology and transmission of HIV-2: why there is no HIV-2 pandemic. JAMA 270: 2083–2086.

García-Lerma G, Yamamoto S, Gómez-Cano M, et al. 1998. Measurement of HIV type 1 plasma virus load based on RT activity: evidence of variabilities in levels of virion-associated RT. J Infect Dis 177:1221–1229.

García-Lerma G, Heneine W. 1999. Analysis of HIV-1 RT activity in plasma: a new tool for the detection of viral variants, virus load measurement, and phenotypic drug resistance testing. AIDS Reviews 1:80–88.

Gao F, Yue L, Robertson D, et al. 1994. Genetic diversity of human immunodeficiency virus type 2: evidence for distinct sequence subtypes with differences in virus biology. J Virol 68:7433–7447.

Gomes P, Costa-Taveira N, Antunes R, et al. 1998. A competitive RT-PCR method for the quantitation of HIV-2 viral load in plasma. 12th World AIDS Conference, Geneva, June 1998, Monduzzi Ed, Milano, 1:145–149.

Guyader M, Emerman M, Sonigo P, Clavel F, Montagnier L, Alizon M. 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) 326:662–669.

Heneine W, Yamamoto S, Switzer W, Spira T, Folks T. 1995. Detection of reverse transcriptase by a highly sensitive assay in sera from persons infected with HIV type 1. J Infect Dis 171:1210–1216.

Heredia A, Vallejo A, Soriano V, et al. 1997. Evidence of HIV-2 infection in Equatorial Guinea (Central Africa): partial genetic analysis of a B subtype virus. AIDS Res Hum Retroviruses 13:439–440.

Heredia A, Vallejo A, Soriano V, et al. 1998a. Phylogenetic analysis of HIV type 2 strains from Portugal. AIDS Res Hum Retroviruses 14:471–473.

Heredia A, Vallejo A, Soriano V, Epstein J, Hewlett I. 1998b. Chemokine receptors and HIV-2. AIDS 11:1198–1199.

Ho D, Mougdil T, Alam M. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 321:1621–1625.

Kanki P, De Cock K. 1994. Epidemiology and natural history of HIV-2. AIDS 8:85–93.

Kanki P. 1999. Human Immunodeficiency Virus type 2 (HIV-2). AIDS Reviews 1:101–108.

Machuca A, Gutiérrez M, Holguín A, Camba M, Belda J, Soriano V. 1998. Seven new cases of HIV-2 infection detected in Madrid in 1996 and 1997. Rev Clin Esp 198:665–668.

Machuca A, Soriano V, Gutiérrez M, et al. 1999. HIV-2 infection in Spain. Intervirology 42:37–42.

Marlink R, Kanki P, Thior I, et al. 1994. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science 265:1587–1590.

Marlink R. 1996. Lessons from the second AIDS virus, HIV-2. AIDS 10:689–699.

Mellors J, Muñoz A, Giorgi J, et al. 1997. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 126:946–954.

Ministério de Saúde. 1999. Comissão Nacional de Luta Contra a SIDA: a situação em Portugal a 30 de Junho de 1999. Doc 116.

O'Brien T, George J, Holmberg S. 1992. HIV type 2 infection in the United States. Epidemiology, diagnosis, and public health implications. JAMA 267:2775–2779.

Peeters M, Koumare B, Mulanga C, et al. 1998. Genetic subtypes of HIV type 1 and HIV type 2 strains in commercial sex workers from Bamako, Mali. AIDS Res Hum Retroviruses 14:51–58.

Quinn T. 1994. Population migration and the spread of types 1 and 2 human immunodeficiency viruses. Proc Natl Acad Sci USA 91: 2407–2414.

Saimot A, Coulaud JP, Mechali D, et al. 1987. HIV-2/LAV-2 in Portuguese man with AIDS who had served in Angola in 1968–74. Lancet i:688.

Santos-Ferreira MO, Cohen T, Lourenco MH, Matos-Almeida MJ, Chamaret S, Luc Montagnier. 1990. A study of seroprevalence of HIV-1 and HIV-2 in six provinces of people's Republic of Angola: clues to the spread of HIV infection. J AIDS 3:780–786.

Simon F, Matheron S, Tamalet C, et al. 1993. Cellular and plasma viral load in patients infected with HIV-2. AIDS 7:1411–1417.

Smallman-Raynor M, Cliff A. 1991. The spread of HIV type 2 into Europe: a geographical analysis. Int J Epidemiol 20:480–489.

Soriano V, Gutiérrez M, Caballero E, et al. 1996. Epidemiology of HIV-2 infection in Spain. Eur J Clin Microbiol Infect Dis 15:383–388.

Switzer W, Wiktor S, Soriano V, et al. 1998. Evidence of *Nef* truncation in human immunodeficiency virus type 2 infection. J Infect Dis 177:65–71.

Takehisa J, Osei-Kwasi M, Ayisi N, et al. 1997. Phylogenetic analysis of HIV type 2 in Ghana and intrasubtype recombination in HIV type 2. AIDS Res Hum Retroviruses 7:621–623.

Van der Ende M, Schutten M, Ly, R. Gruters T, Osterhaus A. 1996. HIV-2 infection in 12 European residents: virus characteristics and disease progression. AIDS 10:1649–1655.

Yamamoto S, Folks T, Heneine W. 1996. Highly sensitive qualitative and quantitative detection of reverse transcriptase activity: optimization, validation, and comparative analysis with other detection systems. J Virol Methods 61:135–143.